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# Evaluation of Capillary Pressure Measurements by Means of an Isotope Tracer Technique

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EVALUATION OF CAPILLARY PRESSURE MEASUREMENTS

BY MEANS OF AN ISOTOPE TRACER TECHNIQUE

by

Marilynn J. Lund

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**LOYOLA UNIVERSITY MEDICAL CENTER**

A thesis submitted to the Faculty of the Graduate School of Loyola  
University of Chicago in Partial fulfillment  
of the Requirements for the Degree of  
Masters in Science

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## BIOGRAPHY

Marilynn Gayda Lund was born on April 15, 1944 in Harvey, Illinois. She attended Bishop Noll Institute in Hammond, Indiana and Loyola University at the Lake Shore Campus, receiving the Bachelor of Science degree in Biology in 1965.

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She married James S. Lund in February of 1967.

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## TABLE OF CONTENTS

Chapter	Page
I. INTRODUCTION.....	1
II. LITERATURE SURVEY.....	
A. Microcirculation Anatomy.....	2
B. Capillary Pressure Measurements.....	8
C. Capillary Filtration Measurements.....	11
D. Isotope Studies.....	17
III. MATERIALS AND METHOD.....	
A. Apparatus and Technique.....	
1. Surgical Procedures.....	19
2. Perfusion Apparatus.....	21
3. Isotope Studies.....	25
4. Counting Apparatus.....	27
B. Experimental Procedures.....	
1. Rest Period.....	27
2. Pressure Alterations.....	28
C. Isotope Technique.....	29
IV. RESULTS.....	
A. Mixing.....	30
B. Isotope Studies.....	32
C. Isogravimetric Determinations.....	35
1. Obtained by lowering arterial pressure first.....	
2. Obtained by elevating venous pressure first.....	

V. DISCUSSION.....	
A. Mixing Apparatus.....	46
B. Equations and Calculations.....	49
C. Isogravimetric Studies.....	55
1. Increased venous resistance.....	56
2. Arterial resistance.....	57
3. Decreased venous resistance.....	60
D. Isotope Studies.....	61
E. Hypothesis.....	62
VI. SUMMARY.....	70
BIBLIOGRAPHY.....	72

## LIST OF FIGURES

## AND TABLES

Figure	Page
1. Diagrammatic arrangement of the microcirculation.....	3
2. Auto-perfusion system.....	20
3. Perfusion apparatus.....	22
4. Comparison of the effectiveness of the mixing systems....	31
5. <u>In Vitro</u> test: Detection of changes in radioactivity levels.....	33
6. <u>In Vivo</u> test: Detection of changes in radioactivity levels.....	34
7. Polygraph record.....	36
8. Comparison between radioactivity levels during four isogravimetric stages produced by decreasing arterial pressure and increasing venous pressure.....	38
9. Comparison between radioactivity levels during four isogravimetric stages produced by increasing venous pressure and decreasing arterial pressure.....	39
10. Relationship between venous pressure and flow as obtained in the isogravimetric state.....	42
11. Relationship between venous pressure and flow as obtained in the isogravimetric state.....	43
12. Changes in total resistance, arterial resistance and venous resistance during the isogravimetric state.....	59

### Table

1. Changes in total resistance obtained during the isogravimetric state (decreased arterial pressure and increased venous pressure)..... 58

## CHAPTER I

### INTRODUCTION

The microcirculatory system is an inaccessible segment of the vasculature because capillary pressure or capillary blood flow cannot easily be measured by direct techniques. Indirect techniques require the isolation of large sections of tissue. However, these sections include other segments of the vasculature, which may have characteristics that differ from that of the microcirculation. Pappenheimer and Soto-Rivera (1948) devised a technique to calculate mean capillary pressure indirectly in an isolated tissue by utilizing inflow arterial pressure, outflow venous pressure, and blood flow. The technique experimentally measured the capillary pressure required to oppose the colloid osmotic pressure of the blood protein (isogravimetric capillary pressure). This capillary pressure value is assumed to remain relatively constant. However, initial data accumulated in the present study revealed that more than one isogravimetric capillary pressure value could be determined.

Therefore, an isotope study was conducted in order to investigate the discrepancy. The purpose of the present study was to reveal whether the observation of more than one isogravimetric capillary pressure was due to the mechanics of the technique itself, or to changes within the state of the arterial or venous systems.



## CHAPTER II

### LITERATURE SURVEY

#### A. Microcirculation Anatomy

1. Fundamental Vascular Pattern: The microcirculatory pattern of mesentery (Chambers and Zweifach, 1944), bat wing (Wiedman, 1963), urinary bladder (Grafflin, 1953), and skeletal muscle (Zweifach, 1955) have been examined. Although each bed is peculiar unto itself, there exists a fundamental vascular pattern common to all areas.

A diagrammatic arrangement according to Zweifach (1949) is outlined in figure 1. The microcirculation begins at the precapillary arterioles, vessels approximately 20 to 30 microns in diameter. These vessels consist of an endothelial tube invested by a single layer of smooth muscle. From such vessels arise the metarterioles, tubes of endothelium surrounded by a single but discontinuous layer of smooth muscle. These vessels may continue directly to form collecting venules or they may give rise to preferential channels. The final subdivision of the vascular apparatus is the true capillary, vessels devoid of smooth muscle, except at their point of origin. This very strategically placed smooth muscle has been called the precapillary sphincter.

The preferential or thorough-fare channel must also be mentioned since it is a morphologically and functionally distinct section of the microcirculation (Zweifach, 1955). This vessel is a branch of the

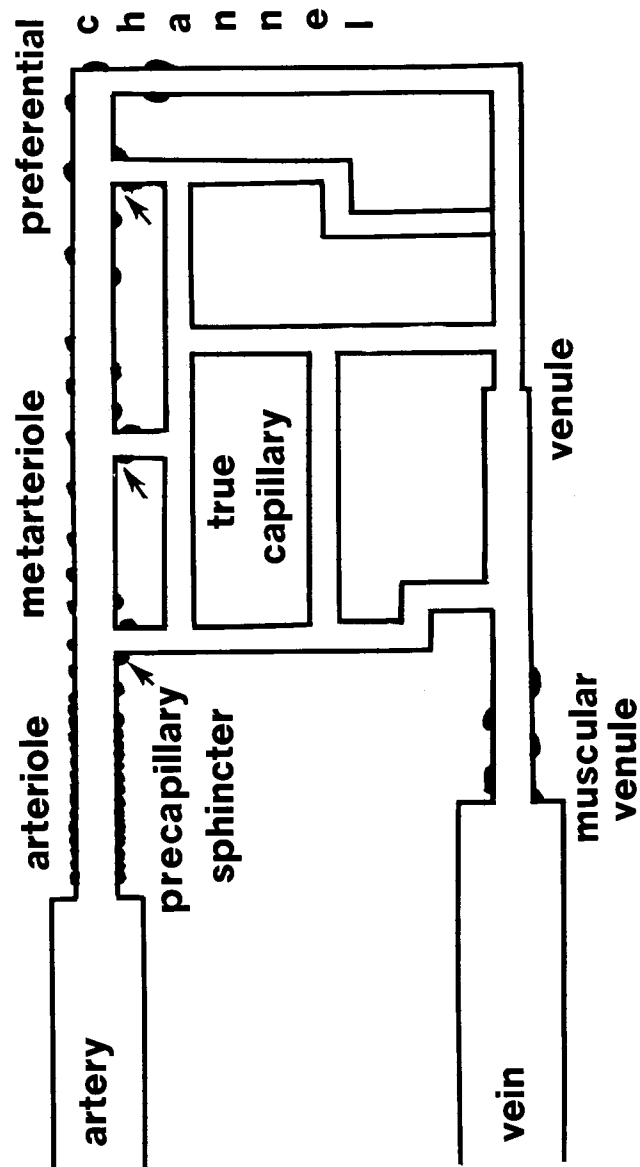


Figure 1. Diagrammatic arrangement of the microcirculation according to Zweifach (1949).

precapillary arteriole, which, as the metarteriole, continues to become the main collection venule. These vessels also have a discontinuous coat of contractile smooth muscle elements at their initial segments, but this coat gradually disappears as the channels join with one another to form collecting venules.

Zweifach (1937, 1949, 1955) differentiated between true capillaries and the non-muscular portions of preferential channels. He called the non-muscular portions of preferential channels muscular capillaries, in spite of the absence of smooth muscle cells. Both vessels are made up of a single layer of endothelium, although muscular capillaries are surrounded by a thick coat of connective tissue. These vessels are similar in that both can function in the transcapillary exchange of fluids. However, muscular capillaries would, because of their thicker coat, normally contribute little to the net transfer of fluids. According to Zweifach (1955), the main distinction between the two vessels is that muscular capillaries provide the most direct blood flow pattern from arterioles to venules. True capillaries arise at right angles, either from the metarteriole or the preferential channel, and the blood flow through these vessels is sporadic and variable in pattern.

Direct microscopic examination of the microcirculation of skeletal muscle has seldom been accomplished. The thick bundles of muscle tissue do not permit such examination. The anatomical arrangement of blood vessels, particularly in man, has been studied by the injection

of radio-opaque substances and the radiographic analysis of the dissected muscle segments (Campbell and Pennefather, 1918). The arterial and venous blood vessels associated with a muscle mass enter at the natural cleavage planes. Each mass is surrounded by a network of vessels, in which the arteries and veins interconnect freely. Muscle fibers are supplied with blood by the branching of metarterioles which distribute to the capillaries. The capillaries run parallel to, and between, the muscle fibers. The vascular pattern has not been described beyond this level of detail.

Zweifach (1955) used rat spinotrapezius muscle in order to perform a microscopic examination of skeletal muscle. The spinotrapezius muscle is a flat, thin, back muscle, which lends itself to such studies. The microcirculation pattern Zweifach described contained all the elements as arranged in figure 1 with one exception. There existed extensive cross connections between small arteries and especially between arterioles to form a series of arcades. This arrangement is not unique and has been described previously by Bloomfield (1945) for a muscle which receives its blood from only one source, entering at the end of the muscle, e.g., the gastrocnemius. Wiedman (1963) also described the arteriolar arcade as a prominent feature of the vasculature in the wing of the common brown bat. Such an arrangement is advantageous since it makes it almost impossible for a capillary network to be isolated from the main blood supply unless an obstruction occurs in the main artery. In vascular beds containing a series

of arcades, the capillary bed is fed by metarterioles which come off at right angles from the arteriolar arcade.

2. Specialized Shunt Mechanisms: There exists within the circulatory system several mechanisms for short circuiting the blood supply from one area to another. According to Boyd (1952) these mechanisms can be classified as (1) arterio-venous anastomoses, (2) specialized muscular apparatus in the walls of blood vessels, and (3) preferential channels.

The first, an arterio-venous anastomoses is a short vessel with well developed muscular elements. It connects arteries and veins without the intermediation of capillaries (Grant, 1930, Zweifach, 1949, Walder, 1952). Clark, who reviewed the literature in 1938, stated that arterio-venous anastomoses normally are found in parts of animals most frequently subjected to mechanical and thermal irritation. These types of stimuli produced local dilatations of arteries and arterioles in areas such as claws and hooves of animals (Grant and Bland, 1931), tips of tails, beaks of birds, and ears of such animals as rabbits. Arterio-venous shunts functioned primarily for temperature regulation in the rabbit (Grant, 1930, Grant, Bland, and Camp, 1932). In man, these shunts are best found in the dermis of the skin, (Grant and Bland, 1931) where they join small arteries to small veins or arterioles with venules.

The distribution of vascular shunts is not limited to the skin

in man. They can be found in a wide range of tissues, including the intestinal tract, glands, liver, genital system, and kidney (Boyd, 1952), but not in skeletal muscle. The studies of Barlow et al (1961) and Renkin et al (1966) did not support the presence of arterio-venous shunts in skeletal muscle. Instead, they suggested that a dual, parallel circulatory system existed within skeletal muscle. One system is a rapidly circulating circuit concerned with the nutrition of muscle fibers, and the second system is a slowly circulating circuit associated with connective tissue.

Boyd's second classification, the specialized muscular apparatus found in the walls of blood vessels will not be discussed extensively. These are, for example, sphincters found in the hepatic veins of the dog, concerned with the regional distribution of blood to that area.

Boyd's third classification, arteric-venous capillaries or preferential channels, have been described for the rat mesoappendix by Zweifach (1940) and Chambers and Zweifach (1944). This vessel was previously described as a further extension of a metarteriole devoid of smooth muscle. Later, Zweifach (1955) noted that these vessels also were present in rat skeletal muscle and were distributed predominately along the free margins of the muscle. During periods of reduced blood flow, the circulation persisted through the preferential channel, while the interior of the muscle fed by capillary vessels became ischemic. No other investigator has described such vessels to date.

## 2. Capillary Pressure Measurement

A. Direct Methods: Capillary blood pressure has been measured directly and indirectly. The direct method is very similar to the technique used to measure arterial or venous pressure in a large vessel. Minute cannulae are inserted into a single capillary vessel and then the pipettes are connected to a water manometer. Carrier and Redberg (1923) were the first to attempt such measurements. Their studies were conducted using human subjects, where the only readily accessible capillary bed is found in the nail fold. They inserted a micro-pipette with a tip diameter of approximately 20 microns. The pressures recorded ranged between 3 to 5 mm Hg. These low pressures do not represent the pressure within a capillary vessel. Carrier and Redberg measured the pressure required to draw blood into the micro-pipette and these pressures are approximately 5 to 10 mm Hg lower, due to the resistance offered by the glass pipette. Furthermore, their pipette size limited them to measuring pressures in venous loops found in the nail bed.

Landis (1926) succeeded in drawing out a finer glass pipette with a tip diameter  $1/6$  to  $1/3$  the length of a frog's red corpuscle, i.e., from 4 to 8 microns." The average pressure he measured in the human nail bed was 20 mm Hg, with a range of 17 to 37 mm Hg. Landis obtained these higher recordings by balancing the pressure in the capillary vessel with the fluid in the manometer. In 1960, MacLeod utilized Landis' method in order to measure capillary pressure

in edematous patients. The control pressure values obtained by direct micropuncture of a single capillary loop in the skin of the nail fold averaged 22 mm Hg, with a range of 16 to 27 mm Hg.

Wiederhielm et al in 1962 obtained a direct recording of capillary pressure in the micro-vessels found in the frog web. An ultra thin micro-electrode, the tip diameter of which was 1 to 5 microns, was filled with a solution that was hypertonic with respect to body fluids. These electrodes were inserted into vessels located in an area magnified with a microscope. The body fluids entered into the tip, diluting the hypertonic solution, and this was recorded as a change in the electrode's electrical resistance. A servo system sensed the resistance change, forced the diluted solution out and recorded the pressure required to maintain the original electrical resistance within the tip. This system, therefore, provided an indication of the pressure of the intracapillary fluid.

Outside of these few instances, direct measurements of capillary pressure has not been reported. For one, the pressure between two individual capillary vessels, close in proximity to each other can vary greatly (Landis, 1934). Periodic alterations in arteriolar resistance results in a continual change in the pressure in an individual capillary (Johnson, 1966). In both man and animals, there are relatively few microcirculatory segments that permit direct cannulation. Thus, technical difficulty and fluctuations in pre-capillary sphincter activity make direct capillary pressure measure-



ments impractical.

B. Indirect methods: Between 1875 and 1924, many attempts were made to measure capillary pressure in man by indirect methods. These methods were all basically similar in that they consisted of an application of a graded pressure upon a tissue by means of a transparent rigid plate (Roy, 1879, Strax and De Graf, 1931) or by means of a rubber sheet surrounding and compressing the tissue under study (Danzer and Hooker, 1920). The indirect method for determining capillary pressure results in a determination of a mean pressure value because it is based upon the entire capillary bed under observation.

In a review, Landis (1934) listed the mean capillary pressures determined by the indirect method as reported by various investigators. Mean capillary pressure ranged from 5 to 45 mm Hg. This wide range is due to the variable criterion taken by each investigator to indicate the state of balance. The average normal values varied so widely for a single tissue that it was impossible to draw any definite conclusions especially concerning the relationship between capillary pressure and the colloid osmotic pressure of the blood.

A second indirect method for measuring mean capillary pressure is the isogravimetric technique developed by Pappenheimer and Soto-Rivera (1948). The technique consists in the lowering of inflow arterial pressure while raising the outflow venous pressure in order to produce a constant preparation weight. Ultimately, a point is

reached where arterial pressure equals venous pressure and flow is zero; the pressure throughout the system is the same. This pressure is called the isogravimetric capillary pressure ( $P_{ci}$ ). However, the isogravimetric capillary pressure determined by the isogravimetric technique is not the same pressure as that which exists under normal in vivo conditions, where arterial pressure is normally high and venous pressure is normally low. Isogravimetric capillary pressure is the pressure required to oppose the colloid osmotic pressure generated by the plasma protein.

### 3. Capillary Filtration Measurement

A. Plethysmograph: Blood vessels are distensible structures. The volume of blood vessels varies in proportion to the amount of blood contained within the intravascular space, and any change in vascular volume will be reflected by a change in the total volume of the organ under study. Lewis and Grant (1925) were the first to employ a plethysmographic technique to study the limb volume changes. They enclosed a human forearm within a glass cone. The cone was closed at each end by rubber tubing and was filled with water. Records were taken of the movements of the water meniscus in the outlet tube by means of the transmission of air to a bellow recorder. Barcroft and Edholm (1943) carried the analysis one step farther. They performed similar experiments but measured the volume changes of the fluid within the cone to indicate blood flow in the human forearm. According

to their method, a cuff was applied to the upper arm in order to impede venous drainage. The venous cuff was inflated and the resulting slope of the limb volume curve was used to calculate the rate of arterial inflow. The volume change continued for approximately fifteen seconds. They explained this as an accumulation of blood, mainly in the venous vessels. However, when the venous pressure rose to equal the occluding pressure applied to the forearm, the veins began to empty; outflow equaled inflow, and the volume of the arm remained constant, except for a slow increase in volume due to the filtration of water into the interstitial space. This continuous increase in volume lasted many minutes. Mc Michael and Morris (1936) calculated the rate of outward capillary filtration from this "filtration slope."

Two problems are involved in the measurement of capillary filtration in a congested limb. There are (1) unknown resting capillary pressure, and (2) unknown venous congestion pressure. Actual venous congestion pressure differs from cuff pressure because of the loss of pressure energy between the cuff and the tissue. To circumvent these problems, Celander and Marild (1962) measured the rates of filtration at 20 and 40 mm Hg cuff pressures. They correlated the difference in filtration rates at these cuff pressures with the difference of venous outflow pressures, produced by cuff pressures of 20 and 40 mm Hg. The difference between the filtration rate at 20 mm Hg and 40 mm Hg was used to calculate the rate of filtration.

They assumed that 80% of the venous pressure increase was transferred back to the capillaries. Thus, the application of a 20 mm Hg venous cuff pressure increase produced a rise of approximately 16 mm Hg capillary pressure. The mean rate of filtration in calf muscles of a newborn child, after a congestion period of 5 minutes, was  $0.011 \pm .004$  ml/ min/ 100 ml/ mm Hg.

The fluctuations in vasomotor tone may produce variations in arm vascular volume. These changes in volume obscure the recording of the filtration slope. In order to avoid these problems, Krogh et al (1932) developed the pressure plethysmograph in order to study filtration rates. By this method, the blood vessels were temporarily emptied by external pressure before and after a period of venous congestion. Quantitative studies of filtration were performed by determining the change in volume of the tissues and the extravascular fluid, but not the contents of the blood vessels. Krogh et al (1932) demonstrated that venous occlusion pressures above 17 cm of water resulted in an exchange of fluid that was directly proportional to the increase in venous pressure. In the light of the work done by Krogh et al, Celander and Marild's filtration rate measurement may represent a constant because they measured a filtration rate above 17 cm of cuff pressures ( at 20 and 40 mm Hg), and were able to express the rate per mm Hg pressure applied.

Krogh, who worked with Landis and Gibbon in 1932, measured a filtration rate of .0055 cc/min/100 cc/cm of water pressure for a 10

minute congestion period. Landis and Gibbon (1933) continued to use the congestion technique with some variations included to improve the apparatus. For periods of congestion between 15 to 30 minutes, the filtration rate was measured to be .0028 cc/min/100 cc of forearm. They showed that the rate of filtration produced by any venous pressure decreased rapidly as fluids accumulated in the tissue spaces over the 30 minute period. This explained the greater rate of filtration previously obtained.

B. Weight Recordings: Danielli (1940), during his study of capillary permeability and edema formation, was the first to periodically measure the weight of the preparation during a perfusion experiment. He used weight changes to quantitate changes in extravascular volume. Hyman and Chambers (1943) placed their muscle preparation on a balance device in order to continuously measure the gain or loss of weight. However, their sensitivity level was low, i.e., a one gram change in weight of the preparation corresponded to a displacement of about one cm on the kymograph drum. Pappenheimer and Soto-Rivera (1948) electrically amplified the sensitivity of the system so that one gram change in weight produced a deflection of 20 to 30 mm on their recording apparatus.

However, weighing devices only can reflect changes in total tissue volume. Pappenheimer and Soto-Rivera (1948) and Johnson (1959, 1960) both agree that the weight change that is recorded is the sum of the increases in intravascular blood volume and interstitial

fluid volume. Since the rate of weight change appeared constant after the first minute, it was assumed that this continuous but slow change represented interstitial fluid accumulation, and that the rapid initial change represented an alteration in intravascular blood volume.

In 1962, Johnson and Hanson collected venous blood samples from isolated sections of perfused intestine. These samples were drawn at various intervals during the course of one weight change and analyzed the changes in plasma protein concentration. Capillary filtration rate calculated from the rate of plasma flow and the change in protein concentration corresponded to the capillary filtration rate as determined by weight change during the slow change phase. Mellander (1960) obtained similar findings for the hindlimb of the cat.

Baker (1964) examined the time course of the weight changes produced by an alteration in pressure and suggested three segments: (1) a rapid increase in weight beginning with the increase in flow and lasting about 7 seconds, (2) a moderate increase in weight starting after 7 seconds and lasting up till 38 seconds, and (3) a gradual increase in weight continuing beyond the 38 second time period. Baker attributed the rapid weight increase, seen in the first interval, largely to intravascular volume change, whereas the gradual but constant weight increase, seen in the third interval, was due wholly to the change of extravascular volume. The moderate weight increase,

seen in the second interval, was thought to be a transition period which included changes in both intravascular and extravascular volumes.

In an attempt to quantitate the relationship between capillary pressure, capillary filtration rate, and effective protein osmotic pressure, Pappenheimer and Soto-Rivera (1948) coupled the technique of weighing the preparation with a technique they termed the isogravimetric method. This ingenious procedure consisted in the lowering of arterial pressure in steps of 15 to 20 mm Hg and elevating venous pressure in order to establish a constant weight (the isogravimetric state) within the perfused, isolated cat hindlimb. The venous pressure required to oppose net reabsorption of fluid between the plasma and tissue, determined at zero flow, was termed the isogravimetric capillary pressure. Ultimately, by using a series of equations, it was possible to estimate mean capillary pressure.

In order to localize the site of autoregulation within the intestine, Johnson and Hanson (1962) used the same technique. This technique was well suited for their purpose since it permitted the separate estimation of arterial and venous resistance. During later studies on mean capillary pressure and venous resistance in the intestine (Johnson, 1965) and skeletal muscle (Thelesius and Johnson, 1966), the procedure was modified so that one major arterial pressure drop and one major venous pressure increase to the point of zero flow was used to determine the isogravimetric capillary pressure.

#### 4. Isotope Studies

Schadle et al (1958) used a radio-isotope technique in conjunction with a weighing technique in order to assess extravascular volume changes. The transcapillary movement of fluid was measured by the dilution of two blood tags, the plasma tag T-1824 and the red cell tag P-32. The tags were added to the blood in the reservoir, stirred, and allowed to flow through their preparation for a period of 15 minutes. The entire venous outflow was caught in interrupted samples. Sample volumes were approximately 1 cc each. These samples were hemolyzed by the addition of a few granules of dry sodium lauryl sulfate. They were then transferred to the multi-celled sample container and one minute beta counts were attained on each sample, using a thin glass-walled Geiger Mueller tube. According to the authors, the technique was sufficiently sensitive to indicate a concentration change of the tags of less than 1%.

However, this study exhibits several weaknesses. Schadle et al used the method as reported by Lawson et al (1954) to determine the concentration of the isotope P-32 within the blood samples. Neither investigator accurately measured the blood sample volumes in order to be sure that they were precisely equal. This is important since the total amount of the isotope contained in a sample will vary as the volume varies. Both reports do not indicate that one cc samples of blood were equivalent to infinite thick samples. If this were the case, precisely measured volumes no longer would be important.



Preliminary studies conducted for this present experiment revealed that 1 cc samples of blood did not produce an infinite thick sample. Moreover, isotopes disintegrate in a random fashion, and thus, high concentrations or extended counting periods are required to obtain the extremely small 1% variation reported for in vitro studies. Neither of these requirements seemed to be met by the investigators. Therefore, it is difficult to accept the findings of the study of Schadle et al.

## CHAPTER III

### MATERIALS AND METHOD

#### 1. Apparatus and Technique

A. Surgical Procedures: The purpose of these experiments was to determine the rate and volume of capillary filtration in the isolated dog forearm by means of a tracer technique. Mongrel dogs were anesthetized intravenously with sodium pentobarbital, 35 mg/ kg, and were treated intravenously with 1000 units/ kg of heparin sodium, prior to cannulation.

A metal clamp, one fourth inch wide, was placed just proximal to the wrist joint and tightened sufficiently to completely occlude the arterial and venous blood flow through the paw. The paw was removed by cauterizing through the carpometacarpal joints or through the antebrachiocarpal joint, the latter of which is located between the distal parts of the radius and ulna. Approximately 80% of the thoracic limb was skinned exposing the entire forearm and the distal portion of the upper arm. The cephalic vein and its accessory veins were ligated at the level of the metal clamp, and the vessel was removed with the skin to the level of the flexor angle of the elbow joint. The cephalic vein of the upper forearm and the median cubital vein were ligated.

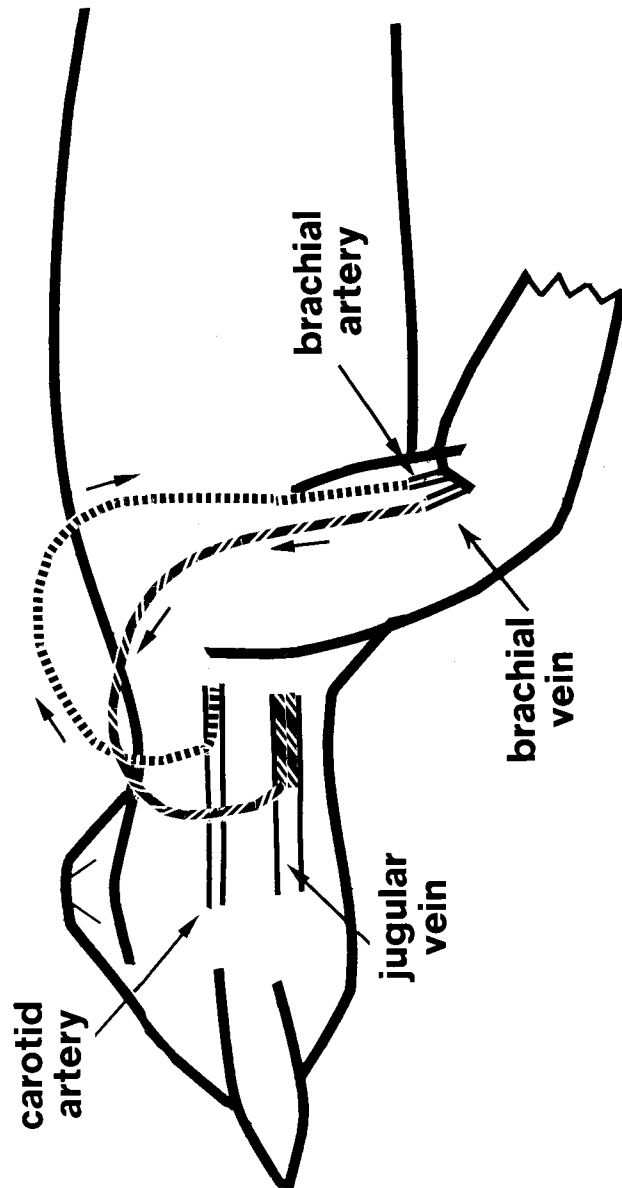


Figure 2. Auto-perfusion system: links the jugular vein to the brachial vein and the brachial artery to the carotid artery.

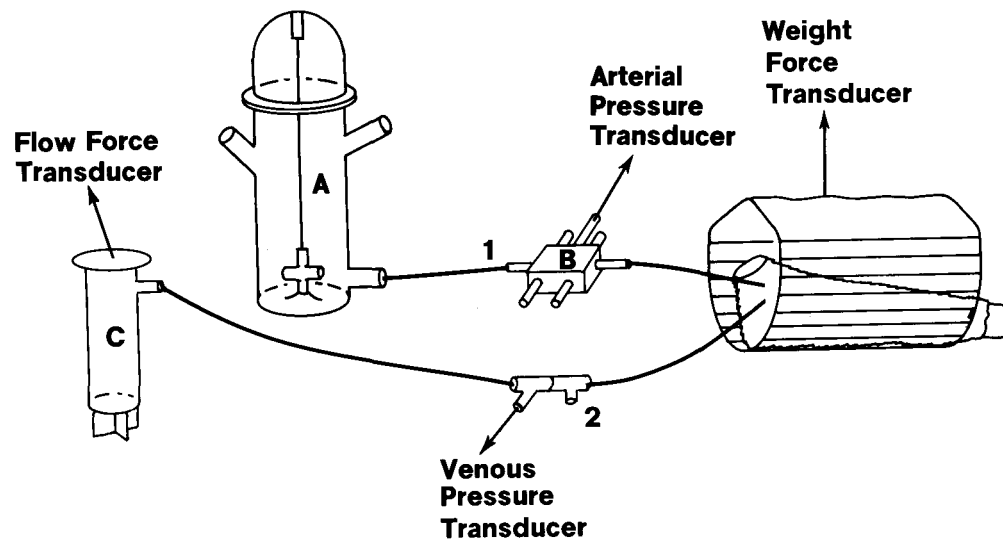
The brachial vein and artery were cannulated proximal to the flexor angle of the elbow joint. The carotid artery and external jugular vein were also cannulated and a closed circuit was established connecting the carotid artery to the brachial artery, and the brachial vein to the external jugular vein (figure 2). By means of this circuit the muscles of the forearm were autoperfused during the remaining surgical procedures.

The forearm was isolated from the animal by cauterizing through the upper arm muscles and sawing through the humerus. Bone wax was used to seal the distal cut end of the humerus.

This preparation, completely isolated from the animal except for the autoperfusion circuit, was coated with light mineral oil to prevent drying. A small surface thermistor was tied to the surface of the muscle and the preparation was surrounded with aluminum foil. A heating coil was wrapped around the outside of the foil covering the forearm. The temperature of the forearm was sensed by a YSI surface thermistor. This was connected to a YSI thermometer-temperature controller, which periodically activated the heating coil in order to maintain the surface temperature at  $37.5^{\circ}$  C.

B. Perfusion Apparatus: Figure 3 is a diagram of the perfusion apparatus used in the experiments. Blood perfusing the tissues was contained in a blood reservoir, a Bellco low-trauma spinner flask with several orifices. A magnetic stirrer with a teflon bar was used

**Figure 3. Perfusion Apparatus.**



**A Pressurized  
Arterial  
Blood Reservoir**

**B Heat  
Manifold**

**C Venous  
Blood  
Container**

**1. Control  
Samples  
Drawn**

**2. Venous  
Samples  
Drawn**

was used to maintain the red blood cells in suspension within the reservoir. One of the orifices was used for a gas inlet and was connected to a tank of compressed 95% O<sub>2</sub> and 5% CO<sub>2</sub>. Pressure delivered to the tissue was controlled by means of a Conoflow H-10XT-H pressure regulator. Lateral pressure of the inflow blood pressure perfusing the artery of the forearm was metered by a Statham P-23-Dc pressure transducer, which was located proximal to the small brachial artery cannula. A Grass Model 7 Polygraph was used to record this data, and all other data including outputs from pressure and force transducers and accessory devices.

The blood was heated just before it entered forearm by passing it through a metal manifold maintained at 37° C by a constant temperature water circulator. The blood was not oxygenated, because it was drawn from the femoral artery of a donor dog. This blood was circulated only once through the preparation.

Venous outflow blood from the brachial vein was collected in a cylindrical container, suspended from a Grass FT 0.03 force transducer. Cumulative blood flow was determined by measuring the weight increase of this container as recorded on a Polygraph channel. When the recording pen deflected to the top of the Polygraph channel, it was electronically rebalanced in order to maintain the pen within the channel. The output from the force transducer supporting the venous collection chamber was differentiated electronically, and this was recorded as blood flow on a separate channel.

Normally, the venous cannula from the forearm was positioned slightly higher than the end of the cannula leading to the collection chamber. In this way the vein was exposed to atmospheric pressure. However, when necessary, the pressure in the collection chamber could be elevated in order to raise terminal venous outflow blood pressure. Near the top of the collection chamber a small adapter was mounted with epoxy through the wall of the chamber. The hole served as a gas inlet and was connected to a pressurized tank of 100% O<sub>2</sub>. A small vent was made near the top of the collection chamber because it was easier to regulate the pressure delivered to the vein when there was a controlled leak in the otherwise closed system. Venous outflow pressure was monitored at a point 7 cm from the brachial vein by means of a Statham P-23-Gv pressure transducer.

The forearm preparation was suspended in a wire cradle from a Grass FT 0.03-B force transducer, counter balanced with a standard 100 gm weight. Under control conditions the combined weight of the forearm, cradle, foil, and heating coil were balanced (equivalent to zero weight) so that only subsequent weight changes were recorded. At the end of each experiment, the perfused skeletal muscle was stripped from the bone and the muscle and its contents were weighed.

The output from the Polygraph channel recording weight was led into a second channel in order to magnify the signal output from the force transducer. A 0.5 gm weight change was equivalent to approximately 30 mm pen deflection. The rate of weight change was easily

determined from this record.

In several cases a dye was added to the blood in the reservoir at the end of the experimental procedures in an attempt to evaluate the extent of the perfusion. Examination of the tissue revealed uniformity of color throughout, even to the small amount of muscle attached to the upperarm.

C. Isotope Studies: Iodine-131 labeled serum albumin (RISA-131, Abbott) was added to the blood in the reservoir. One ml of the labeled albumin (activity: 100  $\mu$ cu/ml) was added per 100 ml of blood, and this provided a final concentration of one  $\mu$ cu/ml of blood. A minimum of one hour mixing time was required to insure a homogeneous distribution of the tag throughout the blood. During preliminary tests, it was found that mixing was incomplete even after one hour. The teflon t-bar stirrer created a vortex within the reservoir resulting in a concentration difference within the container. The concentration of the isotope in the blood samples taken from the outflow cannula increased when the reservoir was filled, one-half filled, and one-fourth filled, respectively. In order to eliminate this concentration difference, a stirrer with prongs arranged along the center glass beam was used. Vertical baffles, which are stationary vertical blades, were placed along the walls of the reservoir. Blood samples mixed in this apparatus exhibited a count per minute rate with a small standard error, indicative of more complete homogeneous mixing.



During an experiment, venous blood samples were collected at a point 7 cm from the forearm. The first three drops of blood were permitted to escape from the collection valve and then a micro-hematocrit tube was filled. This procedure momentarily diverted venous outflow from the collection chamber and was recorded on the polygraph record as a period of zero blood flow.

Control samples, i.e., arterial blood, were drawn from a point proximal to the heat manifold through a three way valve. Three controls were drawn during each experiment; one at the beginning of experimental procedures, a second when the reservoir was half filled with blood, and a third prior to the end of the experimental procedures. A minimum of six hematocrit tubes were filled for each sample drawn.

During experimental procedures, venous blood samples were taken at approximately 15 second intervals. However, in order to obtain the number of samples required for statistical analysis, it became necessary to draw off samples during a steady state condition, i.e., either periods of no weight change or constant weight changes, as determined by the weight channel recordings.

The micro-hematocrit tubes, containing the blood samples, were centrifuged at 5000 revolutions per minute in an Adams Micro-Hematocrit Tube Centrifuge for 7 minutes. The micro-hematocrit tubes were stored under refrigeration prior to plating the blood plasma samples on a planchet. Two, 8 microliter plasma samples were drawn from

each micro-hematocrit tube and each was carefully transferred into the center of a stainless steel planchet. Care was taken during this procedure to maintain a consistent sampling technique. Each tray of 20 planchets was dried by infrared heat for 30 minutes.

D. Counting Apparatus: The counting system consisted of a SC-83 Tracermatic Scaler connected to a SC-86 Tracer Graph Printing Interval Timer. The Geiger Detector, housed in the sample changer unit, was an ultra thin Flow Detector which permitted the radio-assay of isotopes with weak radioactive emissions.

The samples were counted for 100,000 counts. Since the concentration of RISA-131 used in these experiments resulted in an average count of 10,000 counts per minute per 8 microliter sample, about 10 minutes was required to accumulate this count. The half life for I-131 is 8.03 days, and a correction factor was required to account for sample decay which occurred between the onset of counting the samples and the finish. The recorded activity of each sample was therefore corrected at a rate of 0.00357% for each hour of time which elapsed during the counting interval.

## 2. Experimental Procedure

A. Rest Period: The completely isolated forearm was situated within the perfusion apparatus. A rest period of 10 to 30 minutes elapsed during which arterial inflow pressure was set at a non-

oscillating pressure of 100 mm Hg. Venous pressure was not regulated, but the outflow from the veins was exposed to atmospheric pressure.

B. Pressure Alterations: 1) Viability Test: Arterial pressure was increased by a 20 to 25 mm Hg step and maintained for approximately 2 to 4 minutes. Changes in flow and weight were recorded during this procedure. Arterial pressure was then returned to the 100mm Hg level.

2) Isogravimetric determinations: This procedure consisted of altering arterial and venous pressure sufficient to maintain a constant preparation weight. Pressure alterations were performed in either of two sequences: (1) arterial pressure was decreased first and subsequently venous pressure was raised. (2) venous pressure was initially raised after which arterial pressure was decreased. In the first procedure, arterial pressure was decreased in approximately 15 mm Hg steps. Venous pressure was then raised in order to cancel the tendency of the preparation to lose weight, due to the reabsorption of capillary fluid. At each isogravimetric state, the pressures were maintained for a minimum of 30 seconds. The step pressure changes were continued until blood flow decreased to an extremely low level. However, flow was never permitted to stop completely. This low level of flow was accomplished in 4 to 6 paired pressure step changes. After the final step pressure change, arterial pressure was returned to the 100 mm Hg level and venous

pressure in the collection chamber was decreased to zero. In the second procedure, venous outflow pressure was raised in approximately 5 mm Hg steps. Arterial pressure was lowered to a level sufficient to oppose the filtration of fluids within the skeletal muscle and thereby establish the isogravimetric state. Each isogravimetric state was maintained for a minimum of 30 seconds. The step pressure changes were continued until low blood flow levels were recorded. After the final paired pressure changes, the venous outflow pressure was reduced to zero mm Hg and the outflow from the veins was again exposed only to atmospheric pressure. Arterial pressure was returned to and maintained at 100 mm Hg.

3) Isotopes Technique: The same experimental protocol was followed when blood containing RISA-131 perfused the forearm. Similar paired pressure changes were performed, in an alternating manner, with 10 minute rest periods between procedures.

During an isogravimetric state, a maximum of 3 micro-hematocrit tubes were filled, yielding a total of 6 plasma samples. Likewise, 3 micro-hematocrit tubes were filled during the 20 to 25 mm Hg arterial pressure increases (viability test), after one minute had elapsed. The level of radioactivity in the arterial blood (control samples) was measured 3 times during the course of an experiment.

## CHAPTER IV

### RESULTS

A. Mixing: Experiments were conducted to test the efficiency of the mixing system. Blood samples were drawn from the outflow cannula leading to the heat manifold. This cannula penetrated the rubber stopper through the lower orifice of the reservoir. The tip of this cannula, a surgical needle, was bent and positioned towards the center of the reservoir. RISA-131 was added to the top of the blood contained in the Bellco spinner flask. The blood in the reservoir was continuously drained, and samples were taken after one, two, and three hours of mixing.

Figure 4 presents the data obtained in one such test. A comparison was made between blood samples taken from the reservoir while the blood was mixed with (1) the single paddle mixer, and (2) the multiple paddle stirrer with the vertical baffle unit. The blood samples obtained from the reservoir mixed with the single paddle mixer shows an increase in concentration of approximately 8% between the means of samples drawn during the first and third hours. However, the blood samples obtained from the reservoir mixed with the multiple paddle stirrer with vertical baffle unit exhibited a difference within 1.5 % between the means of the three hourly blood samples. This difference was not statistically significant.

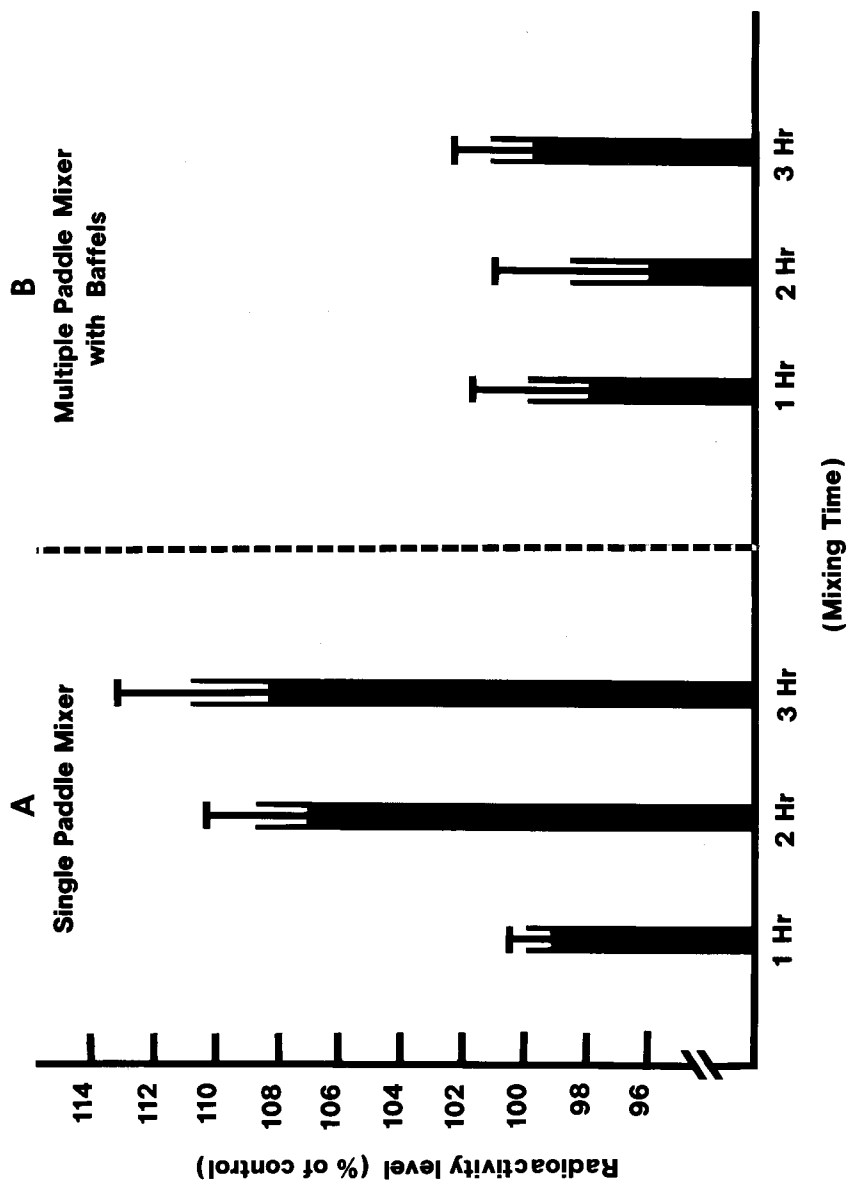


Figure 4. Mixing effectiveness: A) single paddle mixer  
B) multiple paddle system with baffle unit.

B. Isotope Studies: A preliminary study was performed to determine whether or not the method was capable of detecting a change in the concentration of the tagged albumin. A sample of RISA-131 was added to and mixed in the blood contained within a Bellico spinner flask. After one hour, a sample of blood was drawn to serve as a control. The blood was then divided between two reservoirs. A five per cent dilution was accomplished by adding 5 cc of plasma to 95 cc of whole blood, in the second reservoir. Mixing continued in both reservoirs and samples were taken after an additional one and two hours of mixing (designated the two and three hour samples). All samples were prepared and assayed for radio-activity according to methods previously described. The results of this test are presented in figure 5. The five per cent dilution of whole blood produced approximately a ten per cent dilution of the plasma. From the figure, it is quite evident that the method was capable of detecting the dilution.

It is necessary to show that the isotope method was capable also of detecting changes in concentration of the tagged albumin in vivo. Venous blood samples were collected during a period of filtration as detected by weight changes. A comparison between control (arterial) samples and the venous samples is presented in figure 6. The activity level of an 8 microliter venous sample was greater than control samples during a weight

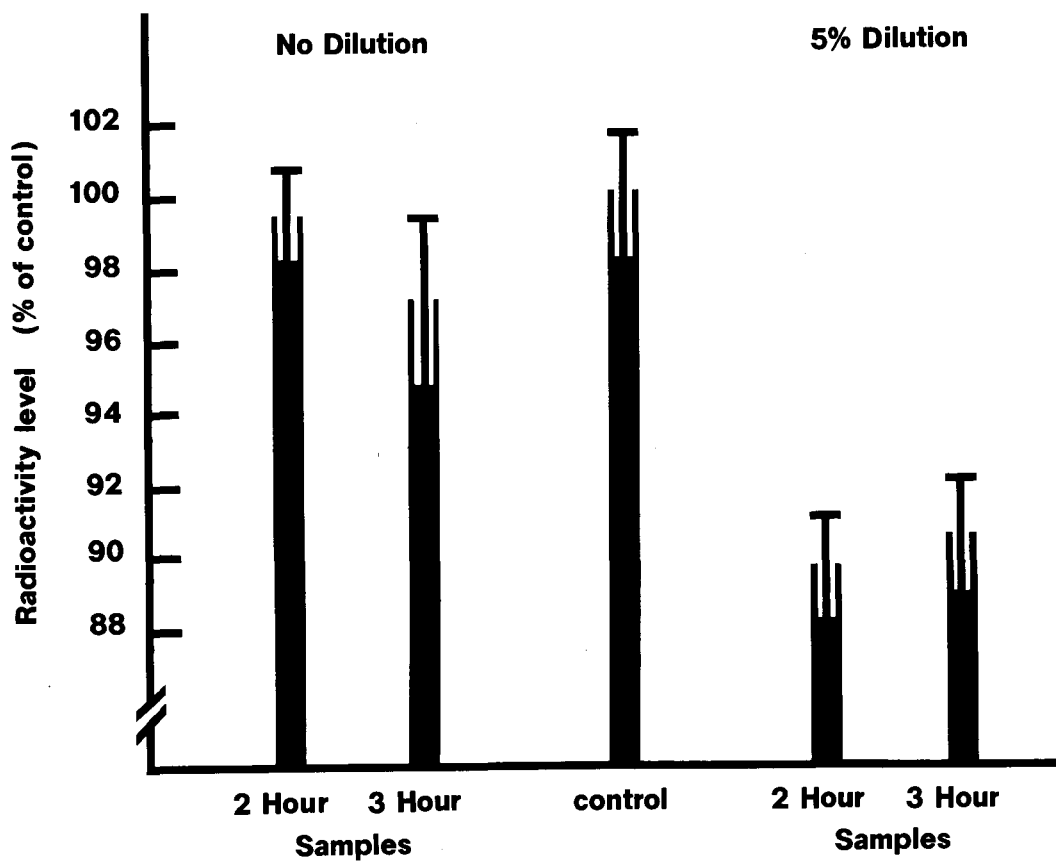
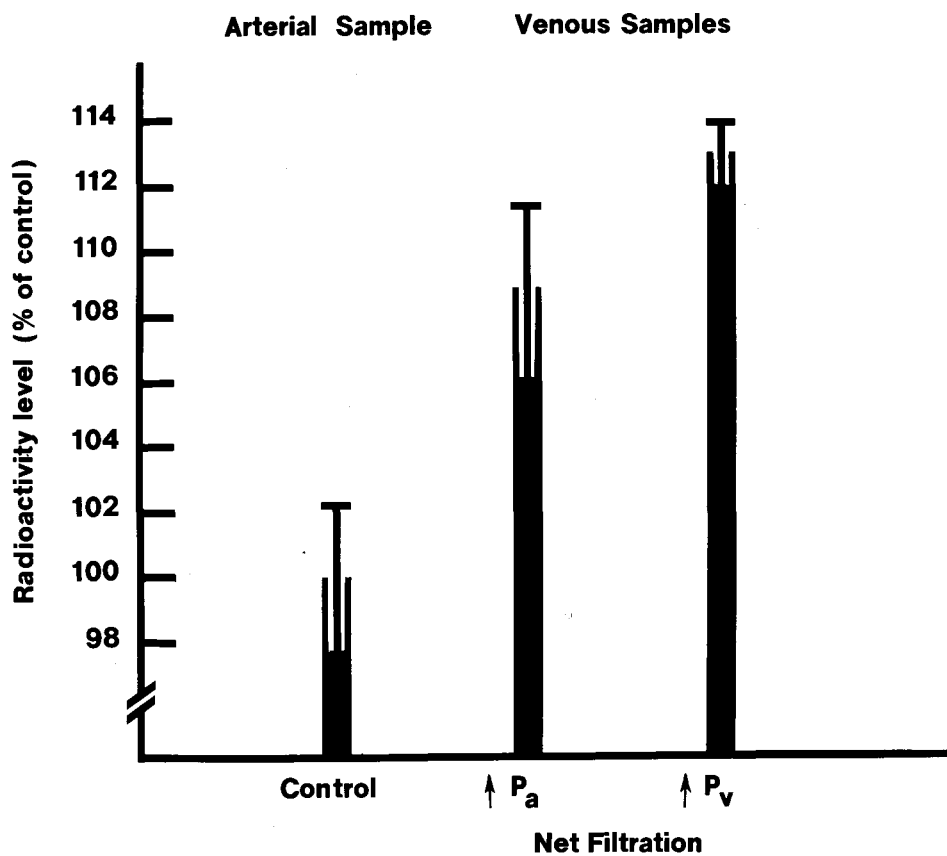


Figure 5.

Radioactivity level of undiluted blood in comparison with the radioactivity level of 5% diluted blood.

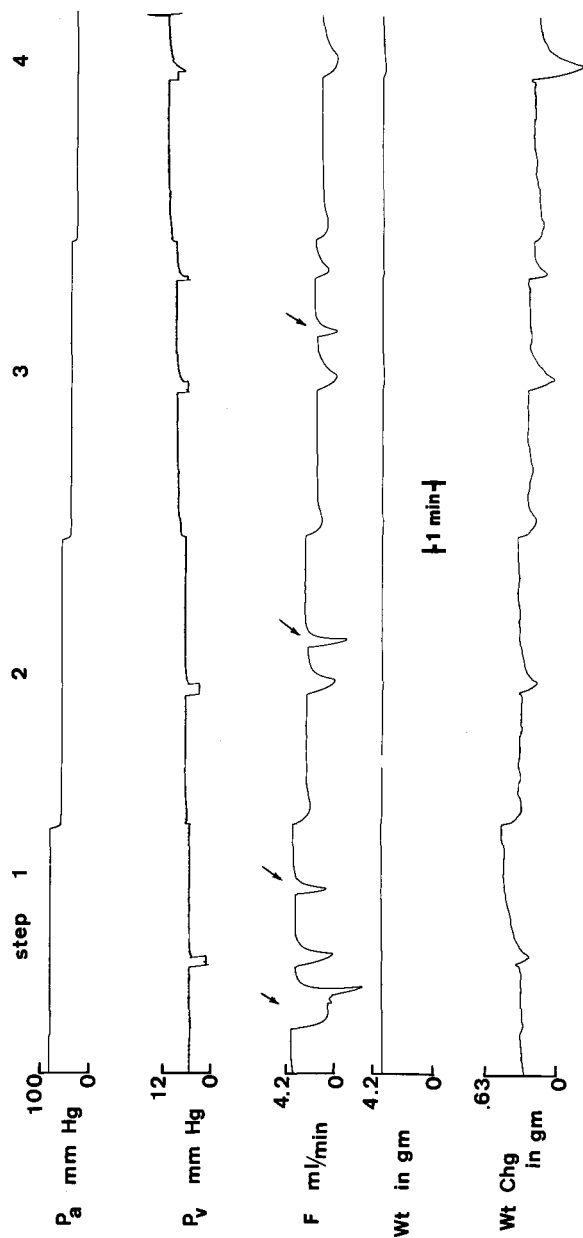




**Figure 6.** Radioactivity level of arterial blood in comparison with the radioactivity level of venous blood collected during two filtration periods.

increase of the preparation. This increase is attributed to a loss of fluid across the capillary membrane, which in effect, concentrated the tagged albumin present in the blood. There is a marked difference between control and venous blood sample radioactivity levels.

C. Isogravimetric Studies: Figure 7 presents a polygraph record illustrating the establishment of four isogravimetric states. The state of no-weight change in each of the four cases is produced by a step decrease in arterial pressure followed by an increase in venous pressure. The rectangular wave which appears in the venous pressure recording, indicates the time at which venous outflow blood was momentarily diverted in order to collect the blood samples. The sampling period was recorded as a drop in pressure, since the sampling point was proximal to the venous pressure transducer. There are four depressions in the flow channel which correspond to the rectangular waves observed in the venous pressure recording. The collection of blood samples momentarily diverted blood outflow from the venous collection chamber, and was recorded as a period of zero flow. A minimum of 30 seconds during each isogravimetric state elapsed before blood samples were taken. The depressions in the flow Channel, marked by arrows, were produced by the automatic rebalancing apparatus. The depressions in the weight change channel



**Figure 7.** Polygraph record illustrating the establishment of four isogravimetric states. The arrows correspond to depressions in the flow recording produced by the automatic rebalancer.

corresponds to the depressions in the venous pressure and flow recordings. During the blood collection period, the veins were exposed to atmospheric pressure. The sudden decrease in weight probably is due to the recoil of the veins following the momentary venous pressure change. This procedure disturbed the isogravimetric state established within the preparation. However, the weight lost was quickly regained and the same isogravimetric state was re-established when the system was again subjected to the elevated venous pressure.

Radioactive samples of arterial blood, which had not passed through the forearm preparation were compared with samples of venous blood collected during periods of no weight change. These periods of no weight change were produced by paired pressure alterations, i.e., by the isogravimetric method. The blood samples were prepared and assayed for radioactivity according to the methods previously described. Figures 8 and 9 present the variation between counts per minute for control (arterial) samples and the venous blood samples, the latter of which were obtained during several isogravimetric experiments on separate preparations. The radioactivity level of venous samples obtained during each isogravimetric state exhibits a variation of 1.5 to 2.3 per cent from the initial control level ( set at 100% ). The mean difference between the control and each of the venous samples is not statistically significant. This data indicates that no net

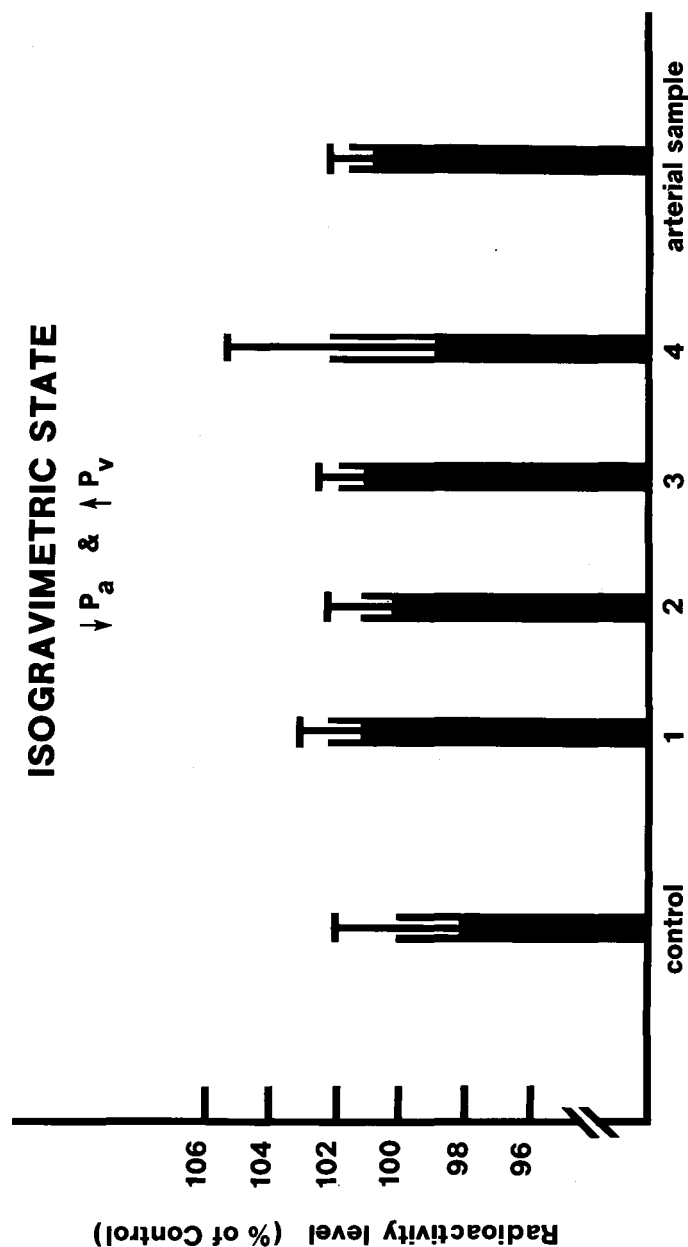
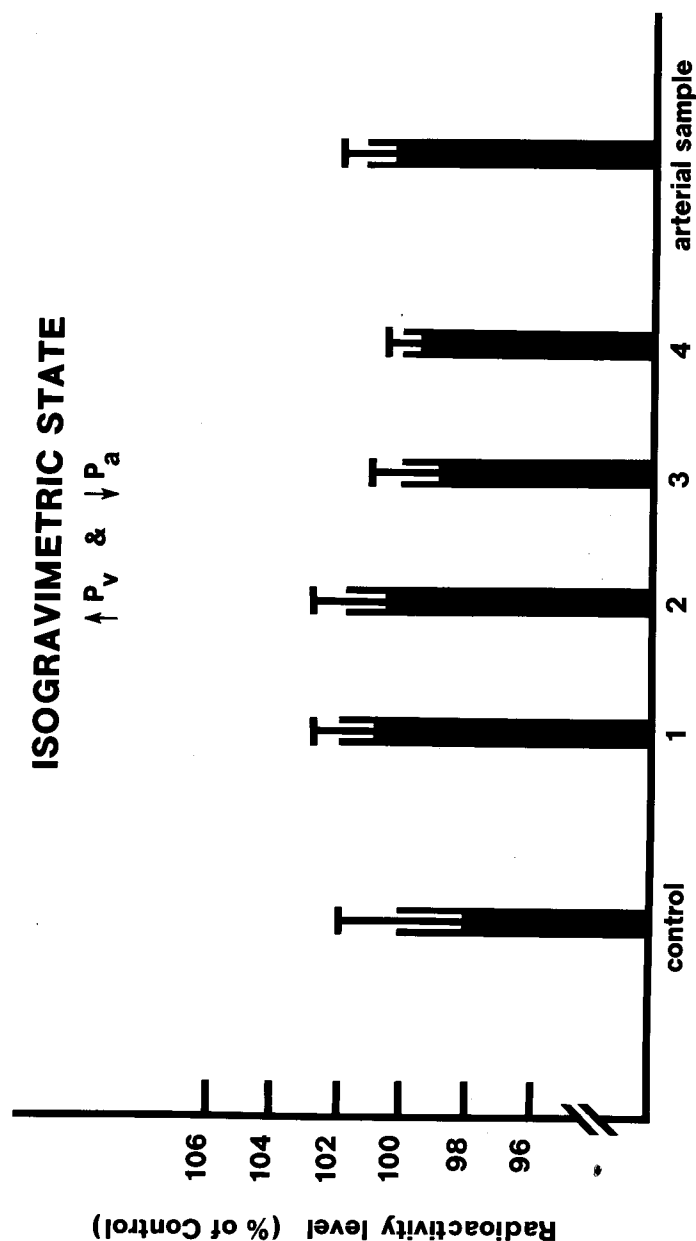


Figure 8.

Variation between radioactivity level of arterial samples and venous blood samples obtained during four isogravimetric stages. Each isogravimetric stage was produced by decreasing arterial pressure and increasing venous pressure.



**Figure 9.**

Variation between radioactivity level of arterial samples and venous blood samples obtained during four isogravimetric stages. Each isogravimetric stage was produced by increasing venous pressure and then decreasing arterial pressure.

filtration was occurring and thus, substantiated the view that the absence of weight change indicates the absence of net filtration.

However, it could be argued from the data presented in figure 8 that net filtration was recorded during the isogravimetric states. The radioactivity level of the venous samples numbers 1,2, 3, and 4 are from 1.5 to 2.3% higher than the initial control level. This per cent change in radioactivity level represents a net filtration rate equal to the minimum filtration rate that could be recorded by the weighing apparatus, i.e., approximately 0.0150 cc/ min equal to a change of one mm/ min. If it is assumed that a net filtration occurred, it would be pertinent to consider the magnitude of the change of inflow arterial pressure or outflow venous pressure that would be required to completely balance net filtration and reabsorption. In figure 6, a filtration of 0.1770 cc/ min was produced by a 2 mm Hg elevation in venous pressure, when arterial pressure was at 80 mm Hg. In another preparation, a filtration rate of 0.1180 cc/ min was produced by a 21 mm Hg elevation in arterial pressure when venous pressure was at 4 mm Hg. From these examples, an estimate of the magnitude change in pressure that would be required can be made. A very small pressure alteration would balance the filtration assumed to be occurring during the isogravimetric states. However, periods of no-weight change were observed for a minimum of 30

seconds, prior to the collection of the venous samples. During that time, no filtration was detected, and thus no pressure correction could be made.

In figure 8, the radioactivity level of the second control (labeled arterial sample) taken immediately after the isogravimetric determination, differed by less than 0.5% from the four venous sample radioactivity levels. It would seem that there was no net filtration recorded by the isotope method during the four cases if they are compared to this arterial sample. Actually, it is more probable that RISA-131 was not completely mixed into the blood during this particular experiment. In that case, the control (arterial sample) drawn immediately following the experimental procedures would be a more accurate estimation of control radioactivity levels than the first control samples, which were drawn approximately one half hour prior to the experimental procedures.

The isogravimetric capillary pressure is defined as the pressure required to oppose the net reabsorption of fluids across the capillary membrane at zero flow. Experimentally, this value was obtained by means of paired pressure alterations which were performed in one of two sequences: (A) arterial pressure was decreased and subsequently venous pressure was elevated sufficient to balance the reabsorptive forces across the capillary membrane and establish a no-weight change or



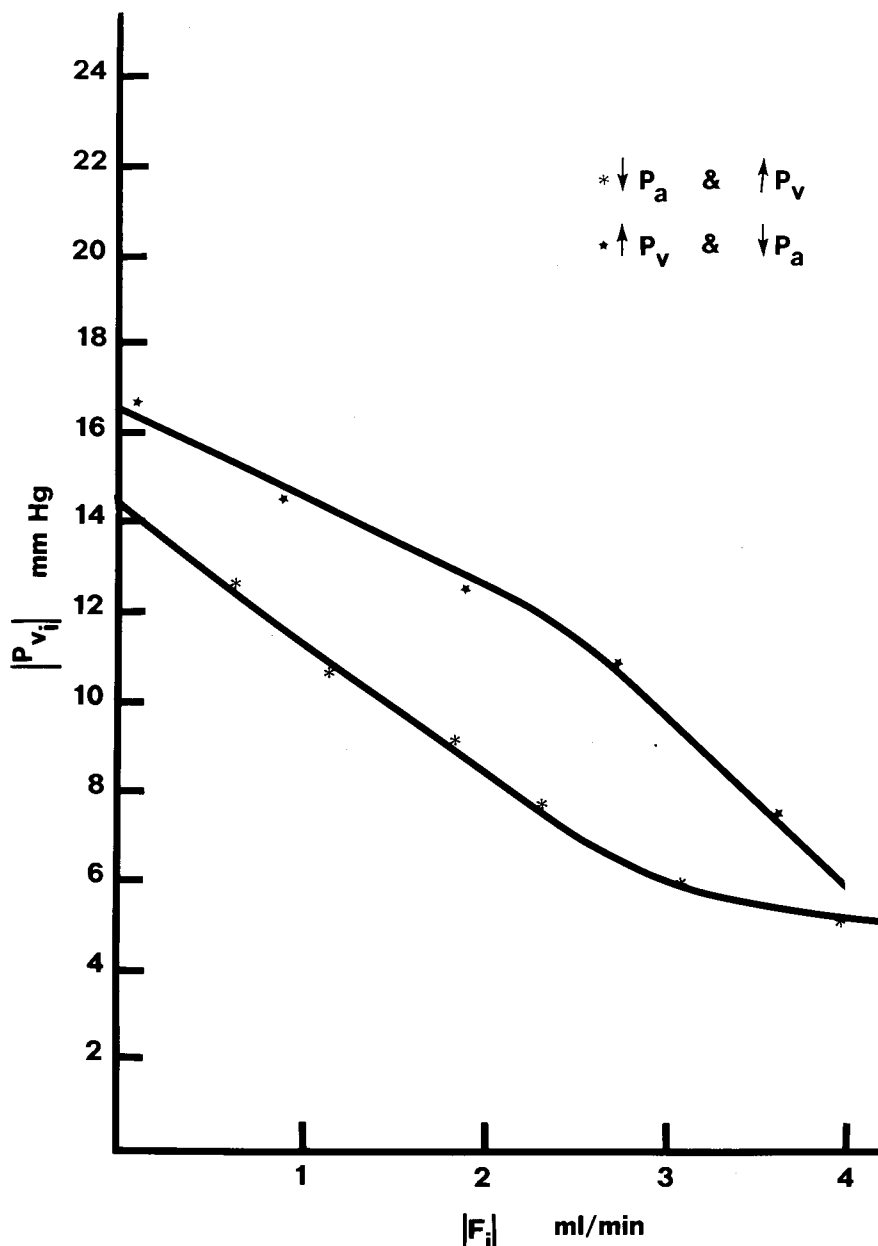


Figure 10. Graph of the equation  $P_{v_1} = P_{c_1} - F R_v$  in which flow is plotted against venous pressure. The intercept value of the ordinate is defined as the isogravimetric capillary pressure ( $P_{c_1}$ ). The slopes of the non-linear lines are an indication of venous resistance ( $R_v$ ).

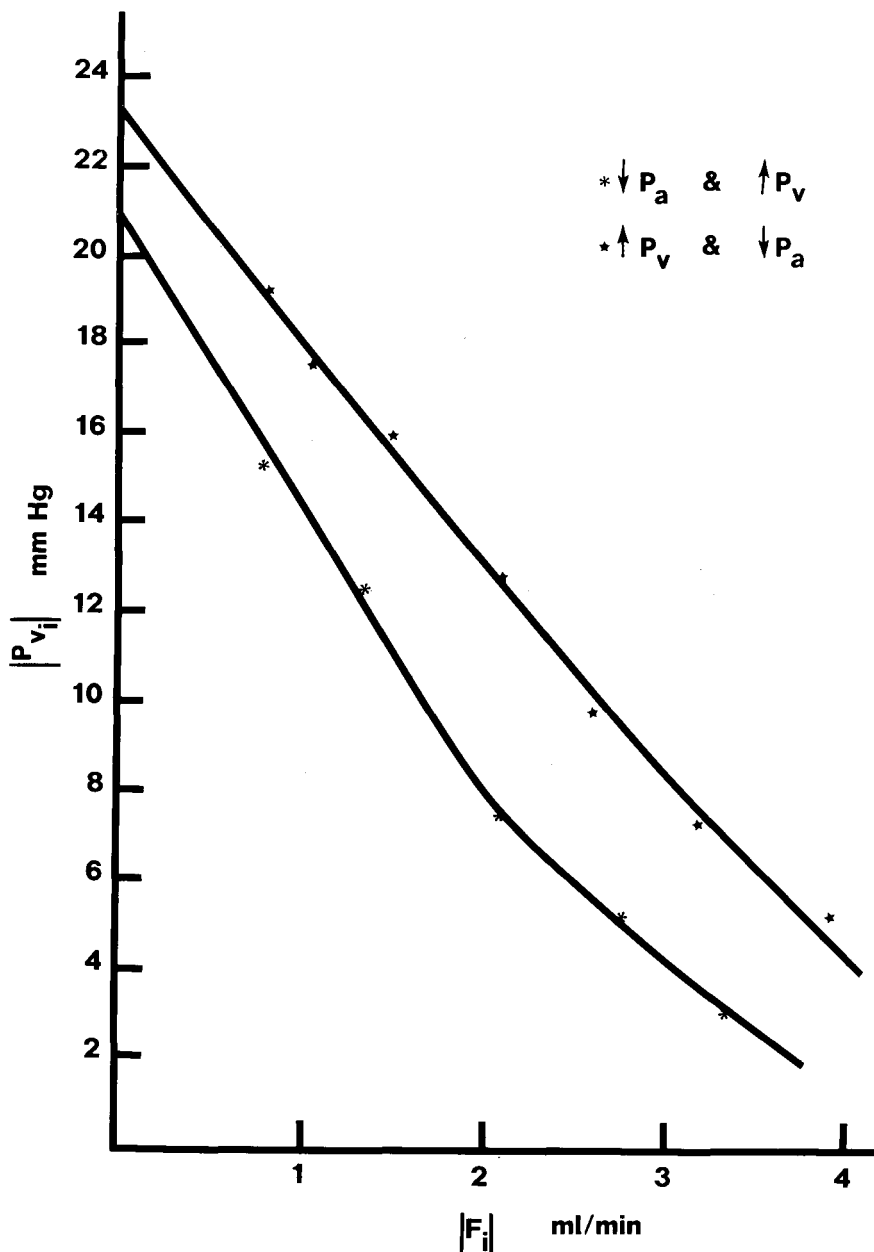


Figure 11. Graph of the equation  $P_{v_1} = P_{c_1} - F R_v$  in

which flow is plotted against venous pressure. The intercept value of the ordinate is defined as the isogravimetric capillary pressure ( $P_{c_1}$ ). The slopes of the non-linear lines are an indication of venous resistance ( $R_v$ ).

isogravimetric state within the forearm preparation. (B) Venous pressure was initially elevated after which arterial pressure was decreased sufficient to balance the filtration forces and again establish an isogravimetric state. Such paired pressure alterations may be referred to as compensated pressure alterations. These compensated pressure alterations in each case were continued until low blood flow rates were recorded. This usually required 4 to 6 step-pressure changes. Figures 10 and 11 present data taken from two different preparations in which the two compensated sequences were performed. In all cases, isogravimetric capillary pressure was obtained by extrapolation back to zero flow. In A of figure 10 and 11, arterial pressure alterations were compensated by an elevation in venous pressure. A non-linear line convex to the flow axis resulted. The slope of this line is an indication of venous resistance, which in this case increased as venous pressure was elevated. In B of figures 10 and 11, venous pressure elevation was compensated by an decrease in arterial pressure. In figure 10-B, a non-linear line concave to the flow axis results. According to the slope of this line, venous resistance decreased as venous pressure was elevated. In figure 11-B, a straight line best related the changes between venous pressure and flow. This indicated that venous resistance remained constant during the entire procedure.

The point at which the curves intercept the venous pressure

axis is a measure of the isogravimetric capillary pressure. In all experiments, a higher isogravimetric capillary pressure value ( $P_{c1}$ ) was obtained by compensated pressure alterations, in which venous pressure was the first pressure alteration.

## CHAPTER V

### DISCUSSION

A. Mixing Apparatus: The main technical problem associated with the isotope studies involved the mixing of RISA-131 which was added to the blood contained within the reservoir. The object was to disperse the tagged albumin within the blood in order to produce a homogeneous concentration. Arterial or venous blood samples could then be drawn, and could be compared with one another in order to assess concentration differences attributed to the loss or gain of fluid across the capillary membrane.

Mixing within a vessel is usually achieved by forced convection. This means that the motion of the liquid produced in the vessel must be intense enough to bring about turbulence (Perry, 1950). In the case of laminar flow, molecules pass between adjacent flow layers only due to Brownian movement, whereas in the case of turbulent flow, the elements not only move in parallel layers but also on erratic paths. Homogeneous mixing is effected by the passing of both molecules and fluid elements, such as red blood cells, from one layer into others.

The first mixing apparatus used, the Bellico-low trauma spinner flask, could be characterized as a paddle-type mixer. It was equipped with a teflon t-bar fastened to a vertical shaft which was rotated axially at the center of the floor of the reservoir. According to

Sterbvacek and Tausek (1965) centrally located mechanical impellers put the entire contents of a vessel into motion. At first only a moderate depression of the fluid level around the shaft occurs, but this gradually deepens and in extreme cases extends to the impeller itself as the speed of stirring is increased. This is a simple description of the formation of a vortex. Mixing by vortex is not very effective because of the differences in angular velocities of the fluid within a cylindrical container. In a vortex there is a velocity gradient established so that the velocity of a fluid at the periphery of a vessel is slower than at the center. A vortex was observed when the single paddle mixer was used in the present experiments. Regardless of the length of time taken to mix the fluid, this motion did not result in a homogeneous distribution of the tagged element. Instead, an increasing concentration gradient resulted between the center of the reservoir to the periphery.

The blood samples providing the data illustrated in figure 4-A were obtained through a cannula positioned to draw blood from the center of the reservoir. This figure compares the blood sample concentrations taken after one, two, and three hours of mixing, while the reservoir slowly drained. The concentration of the isotope between the first to the third hour increased approximately 8%. This attributed to the fact that the blood was drawn from the center of the reservoir for the first hour sample. In contrast, as the reservoir emptied the blood samples taken in the third hour were

more representative of the isotope concentration at the periphery of the reservoir.

The second mechanical mixer was a modification of the first. It consisted of a series of paddles, staggered along the length of the vertical shaft--a pattern similar to a spiral stairway. These rotating paddles exerted a pressure upon the blood, inducing a rotational motion in the liquid. The blood directly in the path of the paddles moved at a faster rate than that lying between. Even so, strata of laminar flow develop, parallel to each paddle (Perry, 1950). Mixing between strata was effected only by the small eddies near the periphery of the mixing arms, and this produces an insignificant transfer between layers (Sterbivacek and Tausek, 1965).

To overcome the stratification of the blood, baffles, located along the walls of the mixing reservoir, were installed. They offered two advantages; (1) the mixer was operated more slowly, reducing the trauma to the red blood cells, and (2) the baffles augmented the vertical discharge of fluid between the layers, increasing the circulation of the blood and introducing turbulence to the otherwise laminar flow patterns. At this point, homogeneous mixing within the reservoir was obtained as evident from the data in figure 4-B. Blood samples now drawn one hour apart, while the reservoir was emptied, exhibited concentration differences of about one percent. This degree of error includes all the variability due to the sampling technique, the efficiency of the counting system, and

the completion of mixing. It represents the lowest level of error that could be accomplished with the present methods.

B. Equations and Calculations: The rate of flow of a fluid through a tube may be related to the driving pressure and the resistance to that flow. This relationship may be expressed as:

$$F = \frac{\Delta P}{R} \quad (1)$$

where,

$F$  = rate of blood flow in ml/ min

$\Delta P$  = pressure difference across a vascular bed in mm Hg

$R$  = resistance to the flow in  $\frac{\text{mm Hg}}{\text{ml/min}}$

Since the flow and pressures across the system can be monitored, this expression may be used to calculate the total resistance of the vascular system.

However, this same relationship is true for any segment of the vascular system as long as the pressure gradient across a given section and the flow through that section is known. For example, the vascular system may be divided into the arterial system and venous system. Arterial vessels, in this case refer to vessels which lie proximal to the capillary bed; venous vessels refer to the vessels which lie distal to the capillary bed. The expression which relates arterial flow, pressure and resistance for the arterial section may be stated as:



$$F_a = \frac{P_a - P_c}{R_a} \quad (2)$$

where,

$P_a$  = inflow arterial pressure

$P_c$  = mean capillary pressure

$R_a$  = resistance offered by the arterial vessels

The relationship between these factors is similar when expressed for the venous side of the vasculature.

$$F_v = \frac{P_c - P_v}{R_v} \quad (3)$$

where,

$P_v$  = outflow venous pressure

$P_c$  = mean capillary pressure

$R_v$  = resistance offered by the venous vessels

These values may be related and restated for parameters obtained in the isogravimetric state. Equation 2 restated for the arterial system during the isogravimetric state is:

$$F_{a_1} = \frac{P_{a_1} - P_{c_1}}{R_{a_1}} \quad (4)$$

Similarly, equation 3 may be restated for the venous system in the isogravimetric state.

$$F_{v_1} = \frac{P_{c_1} - P_{v_1}}{R_{v_1}} \quad (5)$$

It is possible to solve equations 4 and 5 for  $P_{c_1}$ , the isogravimetric capillary pressure. The equations may be restated as:

$$P_{c_1} = P_{a_1} - F_1 R_{a_1} \quad (6a)$$

$$P_{a_1} = P_{c_1} + F_1 R_{a_1} \quad (6b)$$

$$P_{c_1} = P_{v_1} + F_1 R_{v_1} \quad (7a)$$

$$P_{v_1} = P_{c_1} - F_1 R_{v_1} \quad (7b)$$

It may be observed the equations 6b and 7 b are in the form of the general expression  $y = mx + b$ . Venous pressure and flow, obtained during the isogravimetric state (equation 7b) may be described graphically by plotting venous pressure along the vertical axis and  $-F$  along the horizontal axis. The point at which the resulting curve intercepts the pressure axis represents a) the venous pressure at which flow would be zero, and b) an estimate of the isogravimetric capillary pressure,  $P_{c_1}$ . Presentation of these curves (figures 10 and 11) in this paper shall be mirror images of the actual graphs, i.e., the curves seen if a mirror is held on the y-axis. These graphs can be used since only the absolute values of  $P_{c_1}$  and the absolute value of the slope of the curve is required. Previous investigators (Pappenheimer and Soto-Rivers, 1948, Johnson, et al, 1966) presented similar graphs of the relationship between

$P_{v_1}$  and  $F$ , although they did not explain that these were mirror images representations of the true curves, or that they were using the absolute value for Flow. Thus, in order to conform to the literature and also avoid unnecessary confusion which might result from the graph involving a plot of  $-F$ , the absolute value for flow ( $|F|$ ) will be plotted along the vertical axis, and venous pressure along the horizontal axis.

Substitution of the intercept value ( $P_{c_1}$ ) into equation 7a permits the calculation of venous resistance. If this is accomplished for each isogravimetric step in a given preparation, venous resistance during isogravimetric conditions may be determined.

The same graphic representation of equation 6b permits calculation of arterial resistance during isogravimetric conditions. However, once  $P_{c_1}$  is determined, it may be substituted into equation 6a, and it becomes possible to determine arterial resistance for each isogravimetric state.

According to Pappenheimer and Soto-Riviera (1948), venous resistance does not change during compensated pressure alterations. Thus, venous resistance during isogravimetric conditions was expressed as a straight line, in their experiments. Therefore, they assumed that venous resistance was independent of both the blood flow and changes in filtration and absorption of fluids across the capillary wall. On the basis of this assumption, venous resistance would be constant. Mean capillary pressure following an uncompensated

change in arterial or venous pressure was then calculated from equation 3, which may be restated as:

$$P_c = F_v R_v + P_v \quad (8)$$

Since venous resistance was found to be constant during the isogravimetric state, Pappenheimer and Soto-Rivera (1948) applied this value to equation 8, which expresses the relationship between flow and venous pressure no longer obtained by compensated pressure alterations. The mean capillary pressure thus calculated then may be substituted into equation 2, in order to calculate the arterial resistance for that uncompensated pressure alteration.

The work of Pappenheimer and Soto-Rivera implies that venous resistance is not altered by the distension of the veins due to the alteration of venous pressure. In 1966, Johnson et al demonstrated that venous resistance indeed did remain constant in a whole limb preparation during arterial pressure reduction and accompanying venous pressure elevation. However, they found that venous resistance remained constant only when the preparation included both skin and skeletal muscle. It was evident from their pressure-flow studies on the isolated hindlimb, that the skin blood flow accounted for about 70% of the total blood flow. The hemodynamics of the whole hindlimb vasculature therefore was determined primarily by the cutaneous circulation. When the hindlimb preparation was skinned, the relationship between flow and venous pressure was expressed by a

non-linear line, convex to the flow axis. The slope of the curve indicated that venous resistance increased as arterial pressure was decreased and this was compensated for by an elevation of venous pressure.

Although venous resistance in skinned skeletal muscle preparations has been shown to be dependent upon the pressure levels and flow, it is still possible to determine mean capillary pressure and arterial and venous resistances. Isogravimetric capillary pressure can be determined by either extrapolation to the venous pressure axis intercept value, or by performing isogravimetric determinations under conditions of zero flow (Johnson, 1965).  $P_{c_1}$  may be then substituted into equations 6 and 7 in order to calculate arterial or venous resistance under isogravimetric conditions. Resistance values may be calculated using flow, venous pressure or arterial pressure at each step of the isogravimetric procedure.

However, it is more complicated to arrive at resistance changes for an uncompensated state. Since isogravimetric capillary pressure is equal to but opposite in value to the colloid osmotic pressure of the plasma proteins, the mean pressure head across the capillary membrane available for filtration or absorption is determined by the difference between capillary pressure in uncompensated states and isogravimetric states. Pappenheimer in 1948 and Pappenheimer et al in 1952 used this method to calculate the rate of fluid exchange and found that it is independent of the absolute value of capillary

and protein pressures and is dependant upon the difference between the two values. The expression which relates these factors is:

$$+ \text{ Filtration} = K (P_c - P_{c_1}) \quad (9)$$

where,

K = proportionality factor termed the 'filtration coefficient' for the capillary membrane

Since the rate of filtration, F, may be determined from weight recordings,  $P_{c_1}$  may be determined from the isogravimetric state, and K is a value experimentally determined and available in the literature (Pappenheimer et al, 1952); finally  $P_c$ , mean capillary pressure, can be calculated. Further substitution of the value for  $P_c$  into equations 2 and 3 will permit the determination of arterial and venous resistance for uncompensated states.

C. Isogravimetric Studies: The isogravimetric technique was used in the present experiments because it provided a means by which mean capillary pressure could be indirectly determined. With these data, arterial and venous resistance could be individually calculated during any pressure change during an experiment. The capillary pressure determined by this technique is termed the isogravimetric capillary pressure. It is the pressure required to oppose the absorption of fluids within the capillary bed which would occur at zero flow. This pressure is approximately equal to the effective osmotic pressure of the blood proteins, but opposite in value.

(1) Increase in Venous Resistance: Two sets of data were generated depending upon the sequence of compensated pressure alterations. Figures 10 and 11 present the data obtained when arterial pressure was decreased and venous pressure was subsequently increased. The convex non-linear relationship between isogravimetric venous pressure and blood flow as expressed in figures 10-A and 11-A in the forelimb skeletal muscle preparation has been previously reported for hindlimb skeletal muscle (Johnson et al, 1966, Thulesius and Johnson, 1966). This relationship indicated that venous resistance increased during the pressure alterations, particularly in the latter steps of the isogravimetric procedure, when venous pressure was especially high.

This same pattern of venous resistance increase during arterial pressure reduction and subsequent venous pressure elevation has been observed in the intestine (Hanson and Johnson, 1962, Johnson and Hanson, 1962). They attributed the increase in venous resistance to an active constriction of the venules. This increase occurred in spite of the fact that venous pressure was elevated. They suggested that a local arteriovenous reflex was responsible. However, these same observers indicated that an arteriovenous reflex does not fully explain the response seen in skeletal muscle (1966). Neither chronic denervation nor sympathetic blocking agents significantly attenuated the venous resistance response observed in the hindlimb. Johnson et al (1966) also discounted

the possibility that a change in capillary permeability produced by the reduction in flow, could be responsible.

(2) Change in Arterial Resistance: In the experiment in which arterial pressure was decreased and venous pressure was subsequently elevated, Thulesius and Johnson (1966) distinguished between three different types of arterial resistance responses. In type I preparations, arterial resistance declined with each reduction of arterial pressure over the entire range of pressures. This pattern was observed in 25% of the experiments. In type II preparations, there was an initial rise in arterial resistance followed by a pronounced fall as arterial pressure was reduced. This pattern was observed in 50% of the experiments. In type III preparations, there was a progressive increase of arterial resistance over the whole range of pressure reduction. This was observed in 25% of the experiments.

Table 1 lists the data from the present experiments and separates it into similar categories. However, these values are total resistance values. The comparison between the present data and Thulesius' is possible on the assumption that total resistance is influenced predominately by arterial resistance changes. Figure 12 presents arterial resistance changes from an individual case of each type. The fact that arterial resistance in each case follows the changes in total resistance for that type supports the above assumption.



Table 1  
TOTAL Resistance Changes  
During  $\downarrow P_a$  and  $\uparrow P_v$

<u>DECREASED</u> (63%)	<u>INCREASED</u> (7%)	<u>NO CHANGE</u> (29%)
22 to 10	22 to 38	* 7 - 8 - 8 - 6
21 to 12	31 to 40	21 - 22-20 -19
24 to 14		20 - 20-18 - 19
23 to 8		24 - 23-24 - 22
20 to 10		8 - 8 - 8 - 6
23 to 8		43 - 39-34 - 41
15 to 5		
20 to 13		# 21 - 23-22 - 23
28 to 13		14 - 15-14 - 15
46 to 18		
15 to 8		* tendency to decrease
32 to 23		# tendency to increase
30 to 24		
17 to 13		
8 to 2		
4 to 2		

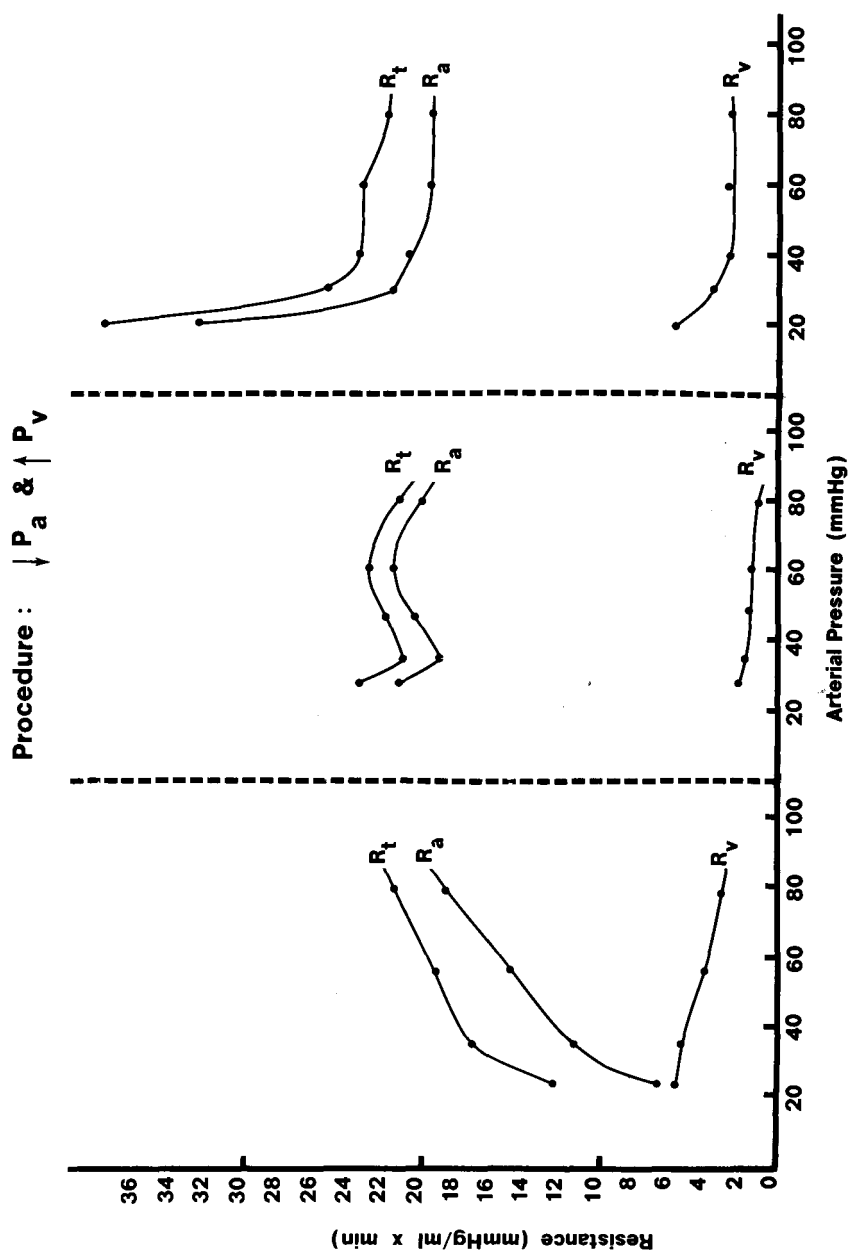


Figure 12. Changes in total resistance, arterial resistance and venous resistance during isogravimetric stages produced by decreasing arterial pressure and increasing venous pressure. The three categories are distinguished according to changes in arterial resistance.

(3) Decrease in Venous Resistance: The non-linear relationship concave to the pressure axis, presented in figure 10-B for isogravimetric venous pressure and flow, has not been previously reported in the literature. A similar resistance change in whole forelimb preparations has been observed by Johnson et al (1966). However, this preparation included skin circulation. In an isolated forelimb preparation, skin accounts for approximately 50% of the total preparation weight. These observers felt that the cutaneous circulation dominated the apparent over all changes in venous resistance; thus the explanation for the decreased venous resistance during venous pressure elevation. Also, the experimental procedure in the present study was not the same as that performed by Johnson et al (1966). These investigators used compensated pressure alterations, but the present study obtained these results when lowering venous pressure and then elevating arterial pressure. Johnson et al obtained their results by performing the opposite i.e., lowering arterial pressure and elevating venous pressure.

In a previous study, Johnson (1965) demonstrated that venous resistance of the intestine decreased when venous but not arterial pressure was changed. Haddy (1964) reported a similar observation in the forelimb. Although the present work refers to compensated pressure changes, venous pressure dominated when it is the first pressure alteration performed.

In addition to the double set of data based upon venous resistance changes, there exists another important difference between the data generated by the two compensated pressure alteration studies. According to figures 10 and 11, the intercept values for both curves are not identical. Early in this discussion, it was assumed that the isogravimetric capillary pressure value, defined as a pressure equal to the protein osmotic pressure of the plasma proteins, but opposite in value, remained constant in an individual preparation. The determination of arterial and venous resistance in the uncompensated state is dependent upon this assumption. However, in all preparations in which total resistance of a preparation remained within the same range during an entire set of experimental procedures, this pattern of dual intercepts values ( $P_{C_1}$ ) was observed.

D. Isotope Studies: Up until now it was accepted that the weighing technique measured (1) vascular volume changes, which lasted up to 40 seconds after a pressure alteration, and (2) net filtration or absorption after 40 seconds elapsed. (Baker, 1964). It was also assumed that a balance between the paired pressure alterations was achieved when the weighing device recorded a no weight change state. However, it is possible that after the 40 second division point, small, intravascular volume changes were also occurring. If this were the case, then the reabsorption of

fluids would be necessary to cancel the increase in intravascular volume, in order to record a state of constant weight. The weighing device would record no weight change, and yet a true isogravimetric state would not have been established.

The isotope studies were performed in order to evaluate this possibility. The present isotope results (figures 8 and 9) which represent the repetition of the isogravimetric experiments with the addition of RISA-131 into the perfusion blood supply, confirmed that no net absorption or filtration were occurring during the period of no weight change. The dual set of data was real and must be explained according to some mechanism inherent within the preparation.

E. Hypothesis: The venous segment is assumed to function as the main blood container within the vascular system and thus to have little importance with respect to flow or total resistance. Although venous resistance is not of the magnitude demonstrated within the arterial system, the venous segment does contribute to the total response. Haddy in 1960 measured arterial resistance (large artery to small artery), small vessel resistance (small artery to small vein), and venous resistance (small vein to large vein). If the assumption, that the pressure at the arteriolar end of the system is 32 mm Hg (Landis, 1929-31), is accepted, it is possible to calculate the resistance across the capillary bed and venules up to a small vein. According to Haddy's data,

this section accounts for approximately 15% of the total resistance offered by the entire vascular system. In comparison, the remainder of the venous system accounts for only 8% of the total resistance. Therefore, venous response, i.e., alterations in resistance to a change in pressure or flow, especially in portions of the venous segment immediately distal to the capillary bed, could significantly alter mean capillary pressure. ( $P_c$ ) and this, in turn, may alter net filtration and absorption.

There are four possible types of behavior that might occur within the venous segment in response to any pressure alteration.

- (1) The veins might act as highly distensible vessels, in which case total venous resistance would decrease in response to an increase in pressure.
- (2) The veins might act as rigid tubes, implying that venous resistance would not change, but remain constant despite changes in pressure.
- (3) The veins might actively oppose pressure changes, which would result in an increased total venous resistance as pressure increases.
- (4) The venous bed might react in any combination of the above three. This type of response would support a double function within the venous segment as suggested by Folkow and Mellander (1964), i.e., an active proximal and passive distal segment of the venous vessels.

The first case (distensible) is supported by Johnson and Selkurt (1958). On the basis of venous pressure-flow studies, they suggested that changes in venous pressure resulted in a myogenic response of the precapillary vessels but not in any venous segment. The veins responded in a passive manner to pressure changes which were delivered from the arterial side of the vascular segment. Similarly, the changes in venous resistance observed in the intestine (Johnson, 1965) and forelimb (Haddy, 1964) for uncompensated increases in venous pressure are in accord with the premise that veins are passively distensible vessels.

The second case (rigid) is supported by Pappenheimer and Soto-Rivera (1948) on the basis of their isogravimetric studies. Venous resistance was found to be independent of pressure and blood flow. However, this has been explained previously to be due to the influence of cutaneous circulation (Johnson et al, 1966).

The third case, i.e., that veins can actively oppose pressure changes, describes the venous resistance change observed in the present experiments, in which arterial pressure decreases were compensated for by increases in venous pressure. Johnson and Hanson (1962) observed a similar reaction in an isolated ileal segment and attributed this response to a local arterio-venous reflex, because denervation, adrenergic blockade, and

nerve (cold) blockade reduced or abolished the venous resistance increase. Rovick (1966) questioned this conclusion because in no case did venous resistance decrease as venous pressure was elevated. If the active component present in the venous response was eliminated, it would be expected that the veins should then act as distensible or passive vessels, which they did not.

The fourth case, i.e., the veins can respond in a manner not clearly indicative of a passive, rigid, or active vessel, was indirectly discussed by Folkow and Mellander (1964). They presented evidence and a hypothesis regarding the local and reflex control of veins. They suggested that the venous segment should be considered in light of its double function. (1) the resistance function, confined primarily to the venules and small veins, which is responsible for post-capillary resistance, and (2) the capacity function, which is confined primarily to the distal venous segment and is responsible for the passive response observed within the venous segment.

The work of Ablad et al (1963) supports this hypothesized double functioning venous segment. They were able to alter the ratio between pre-and-post-capillary resistance by means of chemical agents. Since mean capillary pressure depends upon this ratio of resistances ( Folkow, 1960), a change in either value can significantly change the pressure existing within the



capillary bed, and thus alter net filtration and absorption. Their data revealed that hydralazine decreases the pre-to post-capillary ratio and increased net capillary filtration. They, therefore, concluded that the drug primarily effected the pre-capillary (arteriolar) vessels. Sodium nitrite had a very small effect upon the ratio, and yet dilated the venous segment markedly; in all probability, the post-capillary (venular) segment also would have been dilated. This would explain the slight change in pre-to post-capillary resistance ratio.

In 1966, Rovick concluded that secondary capacity responses in tongue skeletal muscle, produced by uncompensated pressure changes, were active. In one case where arterial pressure was increased, a delayed decrease in capacity was observed in the absence of any accompanying total resistance response. He also reported that this change was accompanied by no apparent change in venous pressure. Consequently, this secondary capacity response must have been caused by active smooth muscle contraction in the venous segment.

The data of Ablad et al (1963) and Rovick (1966) support the presence of both an active, resistance venous segment, probably located in the venules close to the capillary bed and a more distal, yet passive, capacity venous segment. The present data, obtained where the pattern of compensated pressure alterations was reversed, also suggests the possibility of a double function-

ing venous segment.

Reference has been and will be made in this discussion as to the location of the active and passive components within the venous system. This has been done to simplify the concept of an active venous response to pressure alterations and to emphasize the effects an active venous response would have upon mean capillary pressure. This separation of functions does not exclude the fact that venules and small veins also are capacitance vessels. Likewise, it does not exclude the possibility that the smooth muscle in the larger and more distal venous segment could respond in an active manner to pressure alterations.

If this double function does exist within the venous system, it is possible that the isogravimetric capillary pressure determined by the paired pressure alterations would be different from each other. Curve B of figures 10 and 11, describes the case in which venous pressure was the first pressure alteration. This pressure increase transmitted back throughout the vasculature would distend the entire system, not only the distal, passive venous segment but also the proximal, active venous segment. It has been demonstrated (Dobrin, 1968) that the magnitude of contraction in arteries, induced by active smooth muscle is minimal at large vessel diameters and maximal at small vessel diameters. The hypothesis suggests that in this case the effect of the active venous segment was reduced by the initial elevation in venous

pressure. Thus, if the magnitude of response in the active venous segment was minimized, the changes in post-capillary resistance would then be expected to follow the resistance change of a distensible venous system, in response to a venous pressure elevation. The section of the veins, which accounts for approximately  $2/3$  of the total venous resistance, would appear to be responding as a passive segment. Thus total venous resistance would be expected to decrease during this isogravimetric technique. The supposition is supported by the data since the slope of the curve in figure 10-B indicated a decrease in total venous resistance. Figure 11-B indicated that no change in venous resistance occurred during the entire experimental procedure. In this case, the magnitude of the response in the active venous segment was reduced--yet counter-balanced by the magnitude of the response in the passive venous segment. Likewise, the changes in venous pressure would describe more accurately the changes in pressure occurring within the capillary bed, since the active, and thus opposing, segment situated between the capillary bed and the point at which venous pressure is mechanically elevated, is minimized.

By comparison, curves A in figures 10 and 11 describes the case in which arterial pressure was the first pressure alteration. The pressure change was transmitted to the pre-and post-capillary segments. According to the present hypothesis, the post-cap-

illary segment of the veins would have reacted prior to the second pressure change, i.e., the venous pressure elevation. If the smooth muscle in the venules reacted actively and constricted the segment, instead of reacting in a passive manner, during the short interval of decreased arterial pressure, the resistance in this section would increase. The ratio of pre-to post-capillary resistance would increase, and the gradient for net reabsorption would be decreased. A smaller elevation in venous pressure would be required to establish the isogravimetric state at zero flow. Also, it is possible that the increase in venous resistance in this segment could have dominated the changes in total venous resistance. The slope of curve A in figure 10 and 11 indicated that the total venous resistance increased during the entire procedure. The intercept value indicated that a lower isogravimetric capillary pressure was required at zero flow.

If the above hypothesis is true, then the isogravimetric capillary pressure, presented in B of figure 10 will be most representative of the pressure which was defined as equal to the effective osmotic pressure of the plasma proteins, but opposite in value.

## CHAPTER VI

### SUMMARY

Mean capillary pressure and arterial and venous resistance in an isolated, skinned forearm preparation were determined by means of the isogravimetric technique. A dual set of results was obtained depending upon the sequence of compensated pressure alterations performed: (1) venous resistance increased during compensated pressure alterations when arterial pressure was the first pressure to be altered, (2) venous resistance decreased or did not change during compensated pressure alterations when venous pressure was the first pressure to be altered, and (3) isogravimetric capillary pressure, obtained by means of the compensated pressure alteration at the point of zero flow, was always a few mm Hg higher when venous pressure was the first pressure altered. A radioisotope tracer technique was employed in order to assess the absence of net filtration, because zero transcapillary transfer is the basis for the isogravimetric state. The isotope studies confirmed that a balance between filtration and absorption occurred during each isogravimetric state. An hypothesis was proposed in order to explain the data which attributed a dual function to the venous system. This system consisted of an active, proximal and a passive, distal segment of venous vessels. This hypothesis could be used to account for the opposite changes in venous resistance, and especially the

increased venous resistance during an increase in venous pressure. Moreover, an active response to pressure alteration within the venules and small veins could affect mean capillary pressure. This would then explain the calculation of more than one isogravimetric capillary pressure within the same preparation.

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## APPROVAL SHEET

The thesis submitted by Marilyn Gayda Lund has been read and approved by three members of the faculty of the Graduate School.

The final copies have been examined by the director of the dissertation and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the thesis is now given final approval with reference to content, form and mechanical accuracy.

The thesis is therefore accepted in partial fulfillment of the requirements for the Degree of Master of Science.

16 January 1969

Date

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Signature of Advisor